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# A METHOD FOR THE ISOLATION OF TYPHOID AND COLON BACILLI FROM DRINKING WATERS, ETC.

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The problem of separating typhoid bacilli from samples of drinking waters, etc., by a method which shall at the same time be certain, easy, and fairly rapid, has received the attention of numerous investigators. As a result of all their researches we have various modes of isolation and diagnosis of these organisms. Eliminating the processes of "proving" the organism either colon or typhoid bacilli—the difficulty always presented is that of isolating the particular bacillus, and this, I think, is the greatest obstacle which we have to overcome. When these organisms are present only in very minute quantities, accompanied, or perhaps crowded out, by numerous other bacteria, then, indeed, the difficulty of isolation becomes almost insuperable.

Bacteriologists will, I am sure, agree that under such conditions a great deal of time and labor is demanded, and they must often be much surprised by the ease and rapidity with which colon and typhoid bacilli are often diagnosed with certainty by novices.

In my own experience I find that the usual test applied is a "presumptive test," namely, the production of gas in glucose broth, or some modification of this. This is more often than not the extent of the investigations. Now there is no denying the fact that, over and over again, by such incomplete work, an incalculable amount of damage is done—a public or private water supply, for instance, is glibly condemned on such flimsy evidence. The remarks contained in the Report of the Massachusetts State Board of Health, 1903, p. 263-264, suggest the situation admirably.

As regards the various special media which have been recommended for promoting the growth of colon and typhoid bacilli, nothing need be said here. They are all well known and some of them are extremely useful.

Before describing my method of isolation which depends largely upon the motility possessed by these two organisms, reference must be made to one or two bacteriologists who have attempted to avail themselves of this property of movement, viz., Pakes,[1] Cambier,[2] Moore,[3] and Stoddart.[4] They do not seem to have been encouraged by their results and abandoned them as not leading to any practical outcome.

The method which I here put forward is based upon three facts appertaining to colon and typhoid bacilli:

First, their motility.

Second, their power to grow in some medium which possesses a restraining action upon many other bacteria.

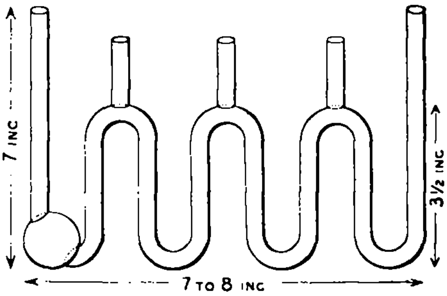
Third, their ability to grow anaerobically.

Expressed briefly the rationale of the work is as follows:

1. The motility will separate the colon and typhoid bacilli from non-motile organisms, and from those less motile than themselves.
2. The restraining medium prevents the ready growth of most organisms except colon and typhoid bacilli:
3. The anaerobic conditions prevent the development of aerobic organisms which form the majority of bacteria with which one comes into contact in ordinary bacteriological examinations.

These three factors when acting together at the same time practically ensure colon and typhoid bacilli having the ascendancy.

The following are the details of the method of applying the principles:



Apparatus for isolating typhoid and colon bacilli.

*Apparatus.* A piece of glass tubing, one-quarter or, better, three-eighths of an inch in diameter, is bent in the manner shown in the accompanying illustration. A bulb is blown near the lower part of the first limb (bulb about 8 or 10 c.c. capacity) and observation openings are made in the uppermost parts of the bent portions.

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[1] Bact. Examination of Water, by Honocks, p. 102.

[2] Revue d'Hygiene, 1902, p. 64.

[3] British Medical Journal, March, 1902, p. 773.

[4] Personal Communication, 1901.

This apparatus is filled with ordinary nutrient broth (*titre*, + 1 per cent. normal acid), to which 0.05 per cent. pure carbolic acid has been added. The openings are plugged with wool and the whole sterilized in Koch's sterilizer for one hour. Inoculations are made at the bulbous end. The tube is then placed beneath a bell jar, in which perfect anaerobic conditions can be assured. This latter point is extremely important because, if not carefully carried out, it will lead to endless failures. The whole apparatus is then placed in the incubator at 37.5° C. At the end of twenty-four, thirty-six, or forty-eight hours samples are taken (about 1 c.c. in quantity) from the observation tubes in order, starting from the one next the bulbous end, and then spread out on Conradi-Drigalski medium. These plates are then incubated at 37.5° C., and during the next twenty-four hours colon and typhoid bacilli will be revealed if present in the original inoculations.

Here I would call attention to another important fact, namely, that typhoid bacilli generally separate from colon bacilli under these conditions. The typhoid bacilli travel faster and farther than the colon bacilli, and usually show up one or two tubes ahead of the colon bacilli. In this manner I have always succeeded in obtaining the typhoid bacilli free from the colon bacilli within forty-eight hours.

A large number of observations have been made extending over many months, with mixtures containing all kinds of bacteria with which one meets, and which the laboratory stock could supply; in no case did I fail to obtain the colon and typhoid bacilli when present. When these two organisms were in minute quantities, as compared with other bacteria, the results were just as favorable.

When using mixtures containing many non-motile forms there was never the slightest difficulty in inducing the typhoid and colon bacilli to free themselves, and in order to put the scheme to severe practical tests, mixtures of all the common motile organisms were generally used.

The actual details of the very numerous experiments under these conditions need not be set forth here as they would only be wearisome to the reader, but as a final statement of the efficacy of this process, I must record the results of one or two experiments where the colon and typhoid bacilli were present only in minute quantities.

Common river water containing the usual varieties of bacteria, was inoculated with typhoid bacilli and from actual counts made the proportion of typhoid bacilli was 350 to the litre. From 50 c.c. of this water the typhoid bacilli were recovered by the process above described.

Colon bacilli were present in the proportion of one bacillus to 1 c.c. of water, and were easily recovered.

As already remarked, in using mixtures of colon and typhoid in minute quantities the typhoid always separated itself from the colon, but both have been recovered.

Having isolated the organisms in this way any confirmatory tests can be applied according to the wishes of the investigator.

In plating out from the experimental tube I have recommended the Conradi-Drigalski medium, and this I do because it offers a great advantage over ordinary agar, especially in the discrimination between typhoid and colon when present together; however, ordinary agar plates will give good results, but the difficulty of discriminating between typhoid and colon when present together on the same plate is much greater under these circumstances. Endo's medium is also a good one to use.

In commenting upon the process for the benefit of others I would give one or two hints regarding some of my failures. Occasionally I have found the ordinary motile organisms, and sometimes true aerobic ones, growing along the whole length of the tube, and in one or two exceptional instances even non-motile organisms. This I discovered to be due to my carelessness in handling the apparatus when putting it into, or taking it out of, the incubator. I found that by turning around quickly with the apparatus in my hands I set up distinct currents of a swirling character inside the tube, and thus brought about a mixing of the contents, which I did not desire. Again, subjecting the apparatus to different degrees of temperature, needlessly repeated, also sets up convexion currents, which sometimes interfere with perfect working. Having put the apparatus in the incubator there is no necessity to take it out until one is actually about to take samples at the end of thirty-six or forty-eight hours, so that one disturbs it only once. If these words of caution are attended to, and these experiments carried out as I have described, I can guarantee successful results to anyone who wishes to investigate them for himself.

Before concluding I should like to add a few words with reference to its application to the bacteriological examination of water. The presence of colon, and perhaps typhoid, bacilli can be demonstrated if they are sufficiently numerous, by direct inoculation with 1, 5, or even 10 c.c. of water. If one wishes to test larger quantities of water for the presence of these two organisms then some process of concentration must be adopted.

Personally I use the well-known one of filtering through a Berkefeld filter, then brushing the organisms into a given quantity of broth or water and inoculating varying quantities of this concentrated mixture, which represents different amounts of original water. I have had extremely good results with this method of isolation when testing samples of natural drinking waters containing a few colon bacilli. Up to the present I have never had the good fortune to come across a sample of natural drinking water contaminated with typhoid, but from the experiments enumerated above, in which I inoculated drinking water with typhoid bacilli in the same proportion as one would expect under natural conditions, I was able to isolate them, and there is good reason to suppose that were we dealing with a natural water also contaminated with typhoid the outcome would be equally successful.

## REPORT OF AN EPITHELIAL LINED FISTULA-IN-ANO IN AN INFANT.

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The special treatises on diseases of the rectum and anus bear witness to the relative scarcity of the fistula-in-ano during infancy, and such cases as are recorded are, from a pathological standpoint, placed in the same category as those encountered in the adult and attributed to like etiological factors, conspicuously tuberculosis.

A number of times I have encountered this condition in infancy and childhood and three times in nurslings. The treatment of these fistulæ calls for radical procedures—division and excision. In their tendency to recur they also partake of the features peculiar to the fistulæ of adolescence.

That tuberculosis alone is not responsible for this lesion the report of the following case suffices:

A male breast-fed infant, aged three months, was brought by its mother, who directed attention to a constant discharge of moisture and feces from a small opening near the anus. Examination: Within one centimetre of the anus there was to be seen a minute opening without any areola or infiltration which betrayed signs of previous inflammation. A No. 1 Bowman probe easily entered the fistula, and its free end, after passing upward for a distance of 1.5 cm., was to be felt by the finger introduced into the rectum, just beyond the internal sphincter. No blood whatsoever appeared after this manipulation.

*Operation.* Following the usual preliminary preparation, and under an ethyl-chloride narcosis, the sphincter was stretched and the fistulous tract divided on a grooved director. It could be seen that the entire tract was lined with a delicate epithelial membrane easily movable over the underlying soft parts. It was excised and the wound allowed to heal by granulation. Complete healing set in within two weeks, and was attended with restoration of the sphincter.

Microscopic examination of the membrane showed structures peculiar to the skin or mucous membrane. There were several superimposed layers of epithelium; those on the surface stratified the deeper ones cylindrical; the papillæ not so marked. The